

Attorney Docket No. 3817.14-1
Customer No. 23308

Amendments to the Claims:

This listing of claims will replace all prior versions, and listing, of claims in the application:

Listing of Claims:

1. (Original): A protein binding assay for measuring IP₃ in a sample employing as reagents a conjugate of IP₃ and a detectable label joined through a bond or linker at the 2-hydroxyl position, and a truncated extracellular portion of an IP₃R having at least about 200 times the affinity for IP₃ than the intact IP₃R, said method comprising:

combining in an assay medium said sample, said conjugate and said binding protein and incubating said mixture for sufficient time for any IP₃ and said conjugate to bind to said binding protein; and

detecting the bound or unbound label as a measure of the IP₃ present in the sample.
2. (Original): A protein binding assay according to Claim 1, wherein said assay is in a homogeneous format.
3. (Original): A protein binding assay according to Claim 1, wherein said sample is a cellular lysate, and wherein said cellular lysate has been treated to block kinases and phosphatases and prepare said sample for said assay.
4. (Original): A protein binding assay according to Claim 1, wherein said binding protein is of not more than about 600 amino acids and comprises at least amino acids 226 – 578 of the mouse IP₃R Type 1.
5. (Original): A protein binding assay according to Claim 1, wherein said label is an enzyme fragment for enzyme complementation.

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6. (Original): A protein binding assay according to Claim 1, wherein said binding protein is a fusion protein of up to about 1.5kD amino acids.
7. (Original): A protein binding assay according to Claim 1, wherein said label is a fluorescer.
8. (Original): A method according to Claim 1, wherein the order of addition of reagents is: (a) combining said sample with said binding protein; and (b) adding said conjugate, with incubating after (a) and (b).
9. (Original): A protein binding assay for measuring IP_3 in a sample using a homogeneous format, employing as reagents a conjugate of IP_3 and an ED of from 37 to 60 amino acids derived from β -galactosidase joined through a linker at the 2-hydroxyl position, and a truncated extracellular portion of an IP_3R having at least about 200 times the affinity for IP_3 than the intact IP_3R , said method comprising:

combining in an assay medium assay components in the following order:
said sample, said binding protein, said conjugate and EA, and incubating
after each combining for sufficient time for complex formation between said
assay components;

adding substrate for said β -galactosidase; and

detecting the turnover of said β -galactosidase of said substrate as a measure
of the IP_3 present in the sample.
10. (Original): A protein binding assay for measuring IP_3 in a sample using a homogeneous format, employing as reagents a conjugate of IP_3 and a fluorescer joined through a linker at the 2-hydroxyl position, and a truncated extracellular portion of an IP_3R having at least about 200 times the affinity for IP_3 than the intact IP_3R , said method comprising:

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combining in an assay medium assay components: said sample, said binding protein, and said conjugate, and incubating for sufficient time for complex formation between said assay components; and

detecting the change in fluorescence polarization as a measure of the IP_3 present in the sample.

11. (Original): A method according to Claim 10, wherein said linker is an aliphatic group of from 4 to 20 carbon atoms.

12. (Currently amended): A method according to ~~Claim 9~~ Claim 10, wherein said fluorescer emits at a wavelength greater than about 500 nm.

13. (Original): A method according to Claim 10, wherein said fluorescer has a polarizability of less than about 60mP.

14. (Original): A protein binding assay for measuring IP_3 in a sample employing as reagents a conjugate of IP_3 and a detectable label joined through a bond or linker at the 2-hydroxyl position, and a truncated extracellular portion of an IP_3R having at least about 200 times the affinity for IP_3 than the intact IP_3R , said method comprising:

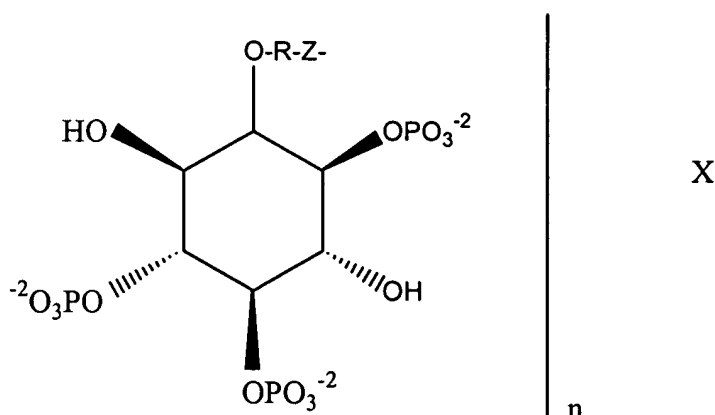
combining in an assay medium said sample, said conjugate, said binding protein and a chemical reductant and incubating said mixture for sufficient time for any IP_3 and said conjugate to bind to said binding protein; and

detecting the bound or unbound label as a measure of the IP_3 present in the sample.

15. (Original): A protein binding assay according to Claim 14, wherein said chemical reductant is a thiol.

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16. (Original): A compound of the formula:



wherein:

R is a neutral linking group of from 4 to 20 carbon atoms bonded to the oxygen through a saturated carbon atom or carbonyl;

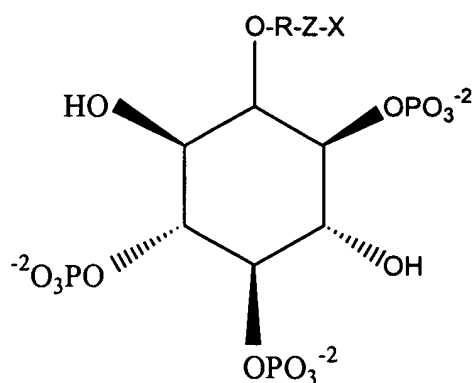
Z is a functionality for linking X to the oxygen at the 2-position;

X is an enzyme donor fragment of β -galactosidase of from 27 to 60 amino acids; and

n is 1 or 2.

17. (Original): A compound of the formula:

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wherein:

R is a neutral linking group of from 2 to 20 carbon atoms bonded to the oxygen through a saturated carbon atom;

Z is a functionality for linking X to the oxygen at the 2-position; and

X is a fluorescer.

18. (Original): A kit comprising a compound according to Claim 17, enzyme acceptor for said enzyme donor and a truncated extracellular portion of an IP₃R having at least about 200 times the affinity for IP₃ than the intact IP₃R.
19. (Original): A kit comprising a compound according to Claim 18, enzyme acceptor for said enzyme donor and a truncated extracellular portion of an IP₃R having at least about 200 times the affinity for IP₃ than the intact IP₃R.
20. (Original): A kit for performing an IP₃ assay comprising a conjugate of IP₃ and a detectable label joined through a bond or linker at the 2-hydroxyl position, a truncated extracellular portion of an IP₃R having at least about 200 times the affinity for IP₃ than the intact IP₃R and instructions for performing said assay.

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21. (Original): A kit according to Claim 20, further comprising a thiol reductant.